

# GASTRO-INTESTINAL IRRITANTS DERIVED FROM STAPHYLOCOCCI

BY

EDWARD ERNEST VICHER

B.S., University of Illinois, 1935

M.S., University of Illinois, 1937

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN  
BACTERIOLOGY IN THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF ILLINOIS, 1942

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## INTRODUCTION

Staphylococci were suggested as playing an etiological role in outbreaks of food poisoning as far back as 1894. It was not, however, until 1930 that the term "staphylococcal food poisoning," as such, came into recognized existence.

It is now known that enterotoxigenic substances are produced by these organisms, more definitely by strains of *Staphylococcus aureus*, in certain foods. The ingestion of this tainted food results in the development of characteristic symptoms of acute illness — nausea, vomiting, and diarrhea. Custard eclairs, cakes, pies, and other bakery goods containing custard or cream filling, as well as such foods as milk and milk products, meats of various kinds, and some poultry salads and gravies have been incriminated.

After the discovery of the role which this group of organisms played, it was only natural that a more thorough study of these food poisoning strains of staphylococci should be carried out. Many investigations have been made as to possible differences between those possessing enterotoxigenic ability and those of non-food poisoning character.

It also follows that the substance or substances produced by the organism and responsible for the gastro-enteric symptoms which result should be studied. Investigations have been carried out in attempts to learn more of the production of these agents by the organism as well as something of their properties, physical, chemical, and otherwise.

## PROBLEM

The purpose of the work undertaken here was to study the various products of staphylococci which may act as gastro-intestinal irritants, including the exotoxin factor termed "enterotoxin" by previous workers, as to their production by the organisms, their properties, and methods of testing for them.

## DISCUSSION AND RESULTS

A variety of animals has been employed in testing staphylococcal filtrates for enterotoxicity. The rhesus monkey and the cat have been most widely used by workers in the field because of the greater consistency with which accurate results have been obtained on these animals. The dog (puppies) and other varieties of monkeys have been employed with some small degree of success. Guinea pigs and rabbits were used by Borthwick (1)

after neutralization of their stomach contents, but the work of this investigator has not been duplicated. Young pigs have recently been suggested as test animals for enterotoxicity.

It would appear from the results of this study that the minute crustacean, *Daphnia magna*, lends itself well to this purpose. This small animal belonging to the family Daphniidae, has been used extensively in bio-assay work on drugs.

The technic, apparatus, and procedure used in this work make the test employing *Daphniae* one which is relatively inexpensive and easy to conduct.

The animal chosen for the test after having been bred according to set standards, must be ten days old and must have a solid-filled gastrointestinal tract. It is then placed into a watertight chamber and an accompanying set-up which allows its perfusion with a solution of the substance being tested. Filtrates of staphylococci, both enterotoxigenic and non-enterotoxigenic, are used in dilutions of 1-50, the original *Daphnia* culture medium being used as a diluent. Controls consist of the original *Daphnia* culture medium as such.

The test is based on the degree of evacuation of waste material present in the tract of the animal at the beginning of the observation period, occurring within one hour.

It was noted that in almost every case in which a positive Dolman reaction (employment of the kitten as test animal) was obtained, the dilution of filtrate used in this test produced a complete evacuation within one hour — in the majority of cases within thirty minutes.

In almost every case in which a negative Dolman reaction was obtained, complete evacuation of the tract did not occur; but on the average, only 10% to 50% of the original contents of the gut were emptied at the end of one hour.

Controls resulted in an average evacuation of from 10% to 50% within one hour.

The results obtained using *Daphnia* as test animal are accurate within the limits of individual variations met with in any animal work.

A study of some of the properties of the exo-enterotoxigenic principle was undertaken.

One of the first of these noted was its relative thermostability. There has been some indecision on the part of investigators as to whether or not enterotoxin may be considered heat-stable. It is the general opinion, however, that it will withstand a temperature of 100° C. without complete destruction. Results obtained in the various phases of this study confirm those of previous workers showing, however, that apparently a gradual decrease in enterotoxic potency does occur on prolonged heat exposure.



Woolpert and Dack (2) investigated to some extent the absorption of enterotoxin by the filtering medium. Although they did not study enterotoxin activity specifically, they found that a Seitz filter pad removed less of this fraction than of the other toxic fractions of staphylococcal filtrates.

In this work, results of treatment of enterotoxin filtrates by intimate contact with the minced Seitz pad itself for varying periods of time showed that absorption is negligible.

Investigations on precipitation of the active enterotoxin principle with ammonium sulfate showed that only at full saturation was it salted out. At 50% and 75% saturation, this factor remains in solution. These results confirm the earlier work of Davison and Dack (3) and the later work of Hammon (4).

It was also found that two volumes of 95% alcohol added to one volume of filtrate resulted in the precipitation of the enterotoxin. Hammon's work (4) on this phase of the problem yielded similar results. The earlier work of Davison and Dack (3) was inconclusive using 50% and 76% alcohol at different conditions of pH, temperature, and time.

A combination of ammonium sulfate and alcohol precipitation procedures resulted in the isolation of a Molisch positive, Biuret negative substance when the simplified acid casein hydrolysate medium of Favorite and Hammon (5) was used. Employment of this method using a semi-solid agar medium yielded inconclusive results because of the carrying over of medium material.

The exo-enterotoxin substance appears to be carbohydrate in nature and is not a protein. Further tests performed by Hammon (4) offer more definite evidence toward this conclusion.

Studies on the endotoxin fractions of certain staphylococci were carried out, employing the technic of Boivin et al. (6). These showed that enterotoxinogenic staphylococci formed and retained within the cell itself an acid-soluble, water-insoluble substance which produced a gastroenteric syndrome in kittens. Such a fraction isolated from non-enterotoxinogenic strains did not appear to possess this ability. It may be suggested that this particular substance is, or may contain, a precursor of the exo-enterotoxin.

## CONCLUSIONS

1. *Daphnia magna*, a minute crustacean which has been used in the bio-assay of various drugs, may be used in testing for enterotoxicity of staphylococcal filtrates. The technic, apparatus, and procedure used in this work make the test relatively inexpensive and easy to conduct.

2. The relative heat stability, yet gradual decrease in potency upon heat treatment, of the enterotoxin has been confirmed by these studies.

3. The absorption of enterotoxin by Seitz filter pads was found to be negligible.

4. Enterotoxic substance can be precipitated out of solution by full saturation with ammonium sulfate. It remains in solution at 50% and 75% saturation.

5. Enterotoxin is insoluble in 95% alcohol. Addition of two volumes of alcohol of this concentration to one volume of filtrate yields a product which possesses definite enterotoxic properties.

6. A highly purified enterotoxic substance can be obtained by combining ammonium sulfate and alcohol precipitation procedures and employing a simplified medium.

7. The enterotoxic substance thus obtained appears to be carbohydrate in nature and not a protein.

8. Enterotoxigenic staphylococci apparently produce an acid-soluble, water-insoluble endotoxic fraction which possesses enterotoxic ability. It may be suggested that this substance is a precursor of the exo-enterotoxin.



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## VITA

Edward Ernest Vicher was born in Chicago, Illinois, November 12, 1914. Received elementary education in Berwyn, Illinois, and attended the J. Sterling Morton High School in Cicero, Illinois. Received B.S. in Pharmacy from University of Illinois in 1935 and M.S. from University of Illinois in 1937. Assistant in Bacteriology, University of Illinois College of Pharmacy from 1935 to 1941; Assistant in Bacteriology, University of Illinois College of Medicine, 1941-1942; Instructor in Bacteriology, University of Illinois College of Medicine, 1942. Member of National Honor Society (J. Sterling Morton High School Chapter), Sigma Xi, Rho Chi, and Phi Kappa Phi.

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